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POTENT CYCLIC UREA HIV PROTEASE INHIBITORS WITH 3-AMINOINDAZOLE P2/P2′ GROUPS

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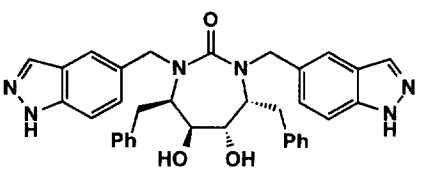
Abstract: Cyclic ureas containing 3-aminoindazole P2/P2′ groups are extremely potent inhibitors of HIV protease. The parent 3-aminoindazole **6** showed a K_i < 0.01 nM but poor translation of enzyme activity to antiviral activity was observed. A series of 3-alkylaminoindazoles revealed that translation improved with increasing lipophilicity. An X-ray crystal structure of **6** bound to HIV protease was obtained.

Over the past decade, intensive research has provided insight into the life cycle of the human immunodeficiency virus (HIV), the causative agent of AIDS. Once the mechanisms for HIV replication were understood, key enzymes were identified which then became targets for rational drug design. Our research efforts have focused on combating AIDS through inhibition of HIV protease, an essential enzyme for viral propagation.[1] Recent clinical trials support using HIV protease inhibitors to fight AIDS by reducing viral load and increasing CD4 cell count.[2]

Previously, we reported that cyclic urea **1** was a potent inhibitor of HIV protease with excellent antiviral activity.[3] Unfortunately, **1** showed low oral bioavailability in rat and dog probably due to poor aqueous solubility (3 ng/mL in water).[4] In this manuscript, we report attempts to improve aqueous solubility and increase the oral bioavailability of **1** by incorporating weakly basic functionality. To this end, a series of 3-aminoindazoles as P2/P2′ groups was investigated (Table 1).



1

Ki = 0.018 nM
IC_{90} = 8 nM
C_{max} = 1.0 uM (dog)
$t_{1/2}$ = 2 h
F% = 13

Synthesis of the parent 3-aminoindazole **6** began with alkylation of urea **2**[5] with bromide **3** to give the bis-alkylated urea **4** (Scheme 1). Bromide **3** was prepared by bromination of 2-fluoro-5-methylbenzonitrile (NBS, $(PhCO_2)_2$, CCl_4, reflux). Conversion of the bis-alkylated urea **4** to the 3-aminoindazole **5** was then accomplished by refluxing with hydrazine. Deprotection with mild acid afforded the parent 3-aminoindazole **6**. Attachment of the methyl and ethyl groups to **5** required the use of Katritzky's benzotriazole chemistry.[6] Attempts to synthesize the methyl and ethyl analogs **7** and **8** using standard reductive alkylation procedures

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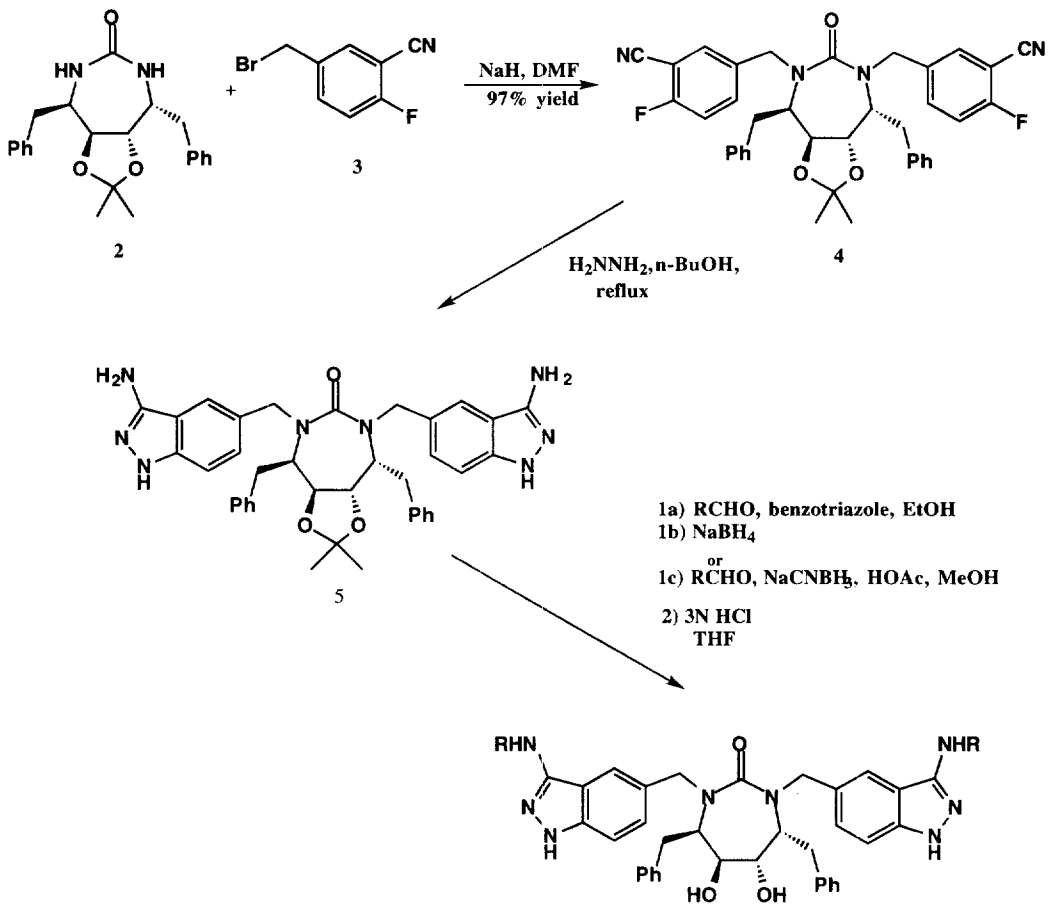
resulted in complex mixtures of alkylation products. However, larger alkyl groups were attached using standard reductive alkylation conditions ($NaCNBH_3$, HOAc, MeOH) to give predominantly the desired monoalkylated 3-aminoindazoles **9–11**. Construction of the pyrrolidine ring of **12** was accomplished by alkylation of **5** with 1,4-dibromobutane.

From Table 1, it can be seen that the parent 3-aminoindazole **6** was an extremely potent inhibitor of HIV protease ($K_i < 0.01$ nM).[7] Addition of the 3-amino group to the indazole ring increased binding over the simple indazole **1**. However, attachment of successively larger alkyl groups resulted in a progressive decrease in activity. Apparently there are unfavorable steric interactions between the alkyl groups and the enzyme.

Scheme 1



In order to understand the binding motif responsible for the excellent potency of the parent aminoindazole, an X-ray crystal structure of **6** bound to HIV protease was obtained (Figure 1). As was observed with our previously reported X-ray of the benzimidazolone **13**,[3] the inhibitor binds symmetrically to the enzyme and the same interactions with the cyclic urea core are present. In addition, the hydrogen bonds from the

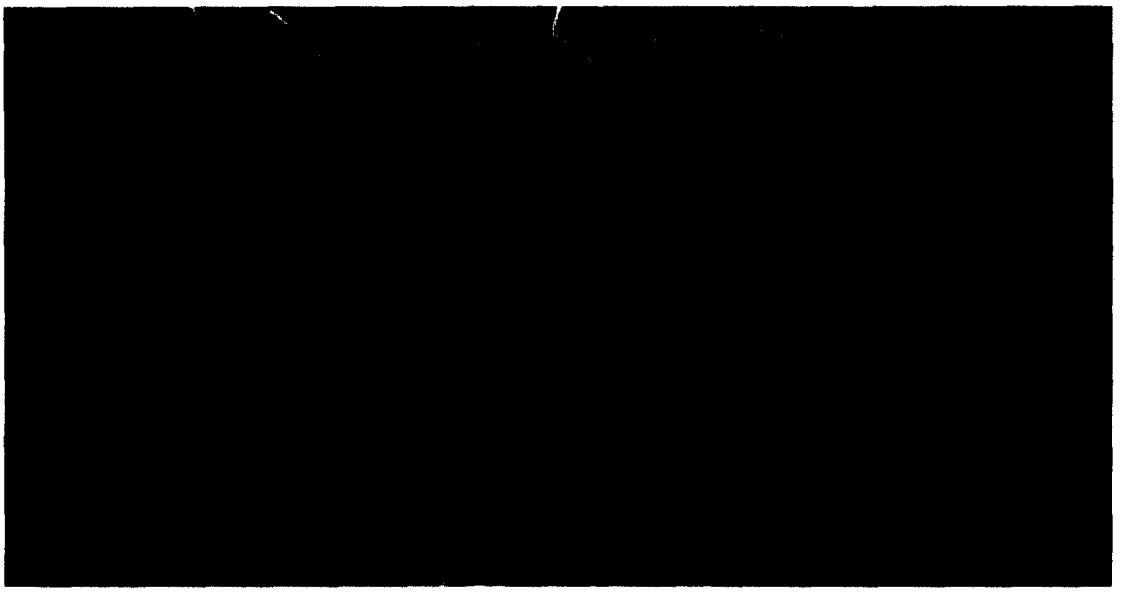


Figure 1. X-ray crystal structure of 3-aminoindazole **6** bound to HIV protease. Hydrogen bonds are shown as dashed lines.

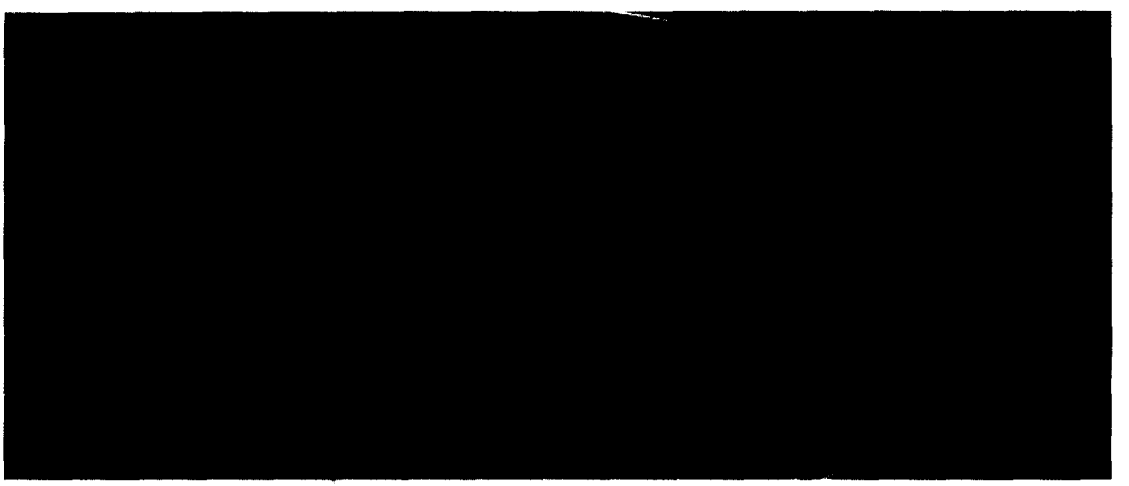
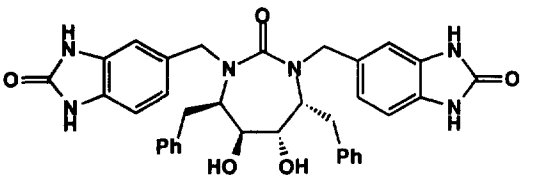


Figure 2. Overlap of X-ray crystal structures of 3-aminoindazole **6** (white) and benzimidazolone **13** (green) bound to HIV protease. Only hydrogen bonded residues of the enzyme to the P2/P2′ groups are shown for clarity.

benzimidazolone carbonyl and N-H of **13** to Asp30 are conserved with the indazole ring of **6** (Figure 2). The N-H of **6** forms a 2.7 Å hydrogen bond to the Asp30 carbonyl and the nitrogen at the two position of **6** forms a 3.2 Å hydrogen bond to the Asp30 N-H. In addition, the -NH_2 at the three position forms two hydrogen bonds to the

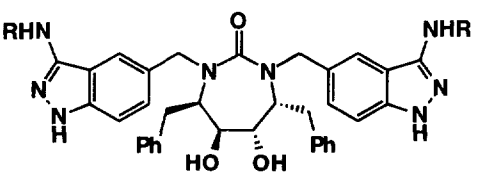


Ki < 0.01 nM
IC_{90} > 65.000 nM

13

enzyme. There is a 3.0 Å hydrogen bond from the -NH_2 to the Asp29 carboxylate side chain and a 3.1 Å hydrogen bond to a water molecule that forms a 2.6 Å hydrogen bond to Gly48 carbonyl. A similar hydrogen bond bridge through water to Gly48 was seen in the X-ray of benzimidazolone **13**. These additional hydrogen bonds to Asp29 and Gly48 account for the increased binding of the 3-aminoindazole **6** over the unsubstituted indazole **1**. In other series of cyclic ureas, we have found that the resistance profile improves with the number of hydrogen bonds in this region of the enzyme.[8] The 3-aminoindazole functionality takes maximum advantage in forming hydrogen bonds and is expected to impart good resistance profiles.

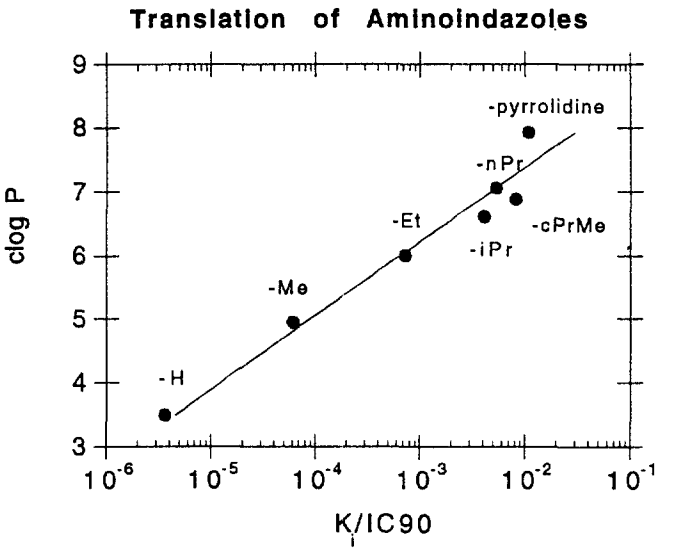
Table 1



Compd	R	K_i (nM)[7]	RNA IC_{90} (nM)[9]	cLog P	-logK_i/IC_{90}
6	H	<0.01	2760	3.5	5.4
7	Me	0.018	279	5.0	4.2
8	Et	0.041	65	6.0	3.2
9	i-Pr	0.09	24	6.6	2.4
10	c-PrCH_2-	0.34	38	6.9	2.1
11	n-Pr	0.23	40	7.0	2.2
12	-$(CH_2)_4$-	1.30	120	7.9	2.0

Although we were able to generate extremely potent inhibitors of HIV protease, this activity did not always translate to antiviral activity in the cell-based assay. In Table 1 we define translation as the negative log of the ratio of the K_i to the IC_{90} (i.e., the smaller the number the better the translation). While 3-aminoindazole **6** (K_i < 0.01 nM) was the most potent compound against HIV protease, it had the worst IC_{90} (2760 nM) in this series. Poor translation from the enzyme assay to the cell-based assay is likely due to problems associated with cell penetration and is commonly observed with polar compounds. For example, the current series showed a very good correlation between translation and cLogP (graph 1); the more lipophilic the compound the better the translation. Unfortunately, as mentioned above, the larger, more lipophilic compounds are worse inhibitors of HIV protease. Increasing the lipophilicity with larger alkyl substituents gave better IC_{90}s in going from methyl to ethyl, to isopropyl or *n*-propyl but further increases in size resulted in worse IC_{90}s. Balancing potency, which comes from having polar H-bond donor/acceptor groups at P2/P2′, with lipophilicity for good translation is thus a significant issue encountered in the design of HIV protease inhibitors.

Graph 1



To investigate if attaching weakly basic groups to indazole **1** (C_{max} = 0.03 ug/mL in rat) would improve oral bioavailabiliy, we tested the ethyl and isopropyl analogs in rat. Unfortunately, both analogs showed no measurable blood levels (<0.01 ug/mL) on oral dosing (10 mg/kg in PEG). It is believed that the high molecular weight and large number of hydrogen bond donor/acceptor groups preclude absorption.

In conclusion, we have discovered that cyclic ureas containing 3-aminoindazole P2/P2′ groups are extremely potent inhibitors of HIV protease. However, the 3-aminoindazole group is very polar and the lipophilicity must be increased for good translation to antiviral activity. Future work will involve the investigation of non-symmetrical cyclic ureas containing only one aminoindazole P2 group for potency. The P2′ group will then be varied to adjust physicochemical properties for better translation and oral bioavailability.

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